2. HEALTH EFFECTS

2.1 INTRODUCTION

This chapter contains descriptions and evaluations of studies and interpretation of data on the health effects associated with exposure to chlorodibromomethane and bromoform. Its purpose is to present levels of significant exposure for chlorodibromomethane and bromoform based on toxicological studies, epidemiological investigations, and environmental exposure data. This information is presented to provide public health officials, physicians, toxicologists, and other interested individuals and groups with (1) an overall perspective of the toxicology of chlorodibromomethane and bromoform and (2) a depiction of significant exposure levels associated with various adverse health effects.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the data in this section are organized first by route of exposure -- inhalation, oral, and dermal -- and then by health effect -- death, systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods -- acute, intermediate, and chronic.

Levels of significant exposure for each exposure route and duration (for which data exist) are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELS) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear, determine whether or not the intensity of the effects varies with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown on the tables and figures may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons or with the identification of persons with the potential to develop such disease may be interested in levels of exposure associated with "serious" effects. Public health officials and project managers concerned with response actions at Superfund sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELS) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels, MRLs) are of interest to health professionals and citizens alike. For certain chemicals, levels of exposure associated with carcinogenic effects may be indicated in the figures. These levels reflect the actual doses associated with the tumor incidences reported in the studies cited. Because cancer effects could exposure levels, the figures also show estimated excess risks, ranging from a risk of one in 10,000 to one in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made, where data were believed reliable, for the most sensitive noncancer end point for each exposure duration. MRLs include adjustments to reflect human variability and, where appropriate, the uncertainty of extrapolating from laboratory animal data to humans. Although methods have been established to derive these levels (Barnes et al. 1987; EPA 1989), uncertainties are associated with the techniques.

2.2.1 Inhalation Exposure

No studies were located regarding health effects of chlorodibromomethane or bromoform in humans following inhalation exposure. In animals, no studies were located regarding effects of chlorodibromomethane, but limited data are available from several older studies on the effects of inhalation exposure to bromoform. These studies are discussed below.

2.2.1.1 Death

Inhalation of very high concentrations (56,000 or 84,000 ppm) of bromoform vapor for 1 hour has been reported to cause death in dogs (Merzbach 1928). The chief symptoms noted were initial excitation followed by deep sedation. This indicates that central nervous system depression is probably the chief cause of death in such acute exposures. Because only two animals were used (one animal per dose) and only high doses were administered, these data do not provide a reliable estimate of the minimum lethal concentration in dogs or other animal species.

2.2.1.2 Systemic Effects

Hepatic and Renal Effects. Only two studies (Dykan 1962; Dykan 1964) were located on the systemic inhalation toxicity of bromoform. These studies (published in Russia and available only as the English abstract) indicate that inhalation exposure of animals to high concentrations of bromoform leads to hepatic and renal injury. Exposure of rats to 240 ppm of bromoform for 10 days resulted in dystrophic and vascular changes in both liver and kidney, with altered renal filtration and hepatic metabolism (Dykan 1964). Longer-term exposure (two months) to concentrations of 24 ppm also lead to hepatic changes (decreased blood clotting and impaired glycogenesis) and renal injury (proteinuria and decreased creatinine clearance) (Dykan 1962). A concentration of 4.8 ppm was estimated to be without significant effects on liver and kidney (Dykan 1964). These changes in liver and kidney appear to resemble the changes produced after oral exposure to bromoform (see Section 2.2.2.2), indicating that bromoform produces similar systemic effects by either route of exposure.

Other Systemic Effects. No studies were located regarding other Systemic effects (respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, dermal/ocular) in animals or humans following inhalation exposure to chlorodibromomethane or bromoform.

2.2.1.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to chlorodibromomethane or bromoform.

2.2.1.4 Neurological Effects

Inhalation exposure to high levels (29,000 ppm or above) of bromoform has been observed to lead to rapid and profound depression of the central nervous system in dogs (Graham 1915; Merzbach 1928). This is presumably due to a nonspecific anesthetic effect similar to that produced by various other volatile halocarbons. Obvious clinical signs included deep relaxation and sedation (Merzbach 1928). Clinical signs of nervous system depression appeared quickly (within minutes), and tended to disappear within a day after exposure ceased (Graham 1915).

No studies were located regarding the following effects in humans or animals after inhalation exposure to chlorodibromomethane or bromoform.

2.2.1.5 Developmental Effects

2.2.1.6 Reproductive Effects

2.2.1.7 Genotoxic Effects

2.2.1.8 Cancer

2.2.2 Oral Exposure

Most information on the health effects of chlorodibromomethane and bromoform comes from studies in animals (rats and mice) exposed by the oral route. For bromoform, there are some observations in humans stemming from the past use of bromoform as a sedative, but no studies were located on the effect of chlorodibromomethane in humans. Summaries of studies that provide reliable quantitative toxicity data are presented in Table 2-l and Figure 2-l for chlorodibromomethane and in Table 2-2 and Figure 2-2 for bromoform. The main conclusions from these studies are discussed below.

2.2.2.1 Death

In the early part of this century, bromoform was often given as a sedative to children suffering from whooping cough, and several deaths due to accidental overdoses have been described (Dwelle 1903; Kobert 1906; Roth 1904 as cited in von Oettingen 1955). The principal clinical signs in fatal cases were those of severe central nervous system depression (unconsciousness, stupor, and loss of reflexes), and death was generally the result of respiratory failure (von Oettingen 1955). If death could be averted, recovery was generally complete within several days (Benson et al. 1907; Burton-Fanning 1901; Kobert 1906).

The dose needed to cause death in children is not known with certainty, but both Dwelle (1903) and Roth (1904) estimated that a dose of about 5 g had been fatal. For a 10 to 20-kg child, this corresponds to a dose of around 250 to 500 mg/kg.

In animal studies, estimates of the acute oral LD_{50} for chlorodibromomethane and bromoform typically range between 800 and 1,600 mg/kg (Bowman et al. 1978; Chu et al. 1982a). Single oral doses as low as 300 to 600 mg/kg can cause death in a few animals (NTP 1985, 1988), quite close to the estimated lethal dose in humans (above). Doses below 250 mg/kg usually do not cause death in animals, even when exposure is continued for 14 to 90 days (Condie et al. 1983; Munson et al. 1982; NTP 1985, 1988).

The cause of death following acute oral exposure of animals has not been thoroughly investigated, but as in humans, the chief clinical signs observed are those of central nervous system depression (Bowman et al. 1978). While central nervous system depression probably is an important factor in acute lethality, in some cases death did not occur until several days after an acute exposure (Bowman et al. 1978; NTP 1985,1988). This suggests that other effects (e.g., hepatic and/or renal injury) may also be important (see Section 2.2.2.2). This is supported

TABLE 2-1. Levels of Significant Exposure to Chlorodibromomethane - Oral

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
ACUTE EXPOSURE								
Death								
1	Rat	(G)	1 d				1186 (LD50 male) 848 (LD50 female)	Chu et al. 1982a
2	Rat	(G)	1 d		310		630 (1 of 10 died)	NTP 1985
3	Rat	(G)	14 d 1x/d		250		500 (8 of 10 died)	NTP 1985
4	Rat	(G)	1 d		1220		1840 (1/5 died) 2650 (LD50 male)	Hewitt et al. 1983
5	Mouse	(G)	1 d				800 (LD50 males) 1200 (LD50 females)	Bowman et al. 1978
6	Mouse	(G)	1 d		160		310 (1 of 10 died)	NTP 1985
7	Mouse	(G)	14 d 1x/d		250		500[a] (7 of 10 died)	NTP 1985
Systemic								
8	Rat	(G)	14 d 1x/d	Renal	250	500 (darkened medullae)		NTP 1985
9	Rat	(G)	1 d	Hepatic		2450 (incr. ser. enz.)		Hewitt et al. 1983
10	Rat	(G)	1 d	Hepatic	1500			Chu et al. 1982a
11	Rat	(G)	1 d	Renal	1500			Chu et al. 1982a
12	Rat	(G)	1 d	Hepatic	1220			Plaa and Hewitt 1982a
13	Rat	(G)	14 d 1x/d	Hepatic	250	500 (mottled liver)		NTP 1985

TABLE 2-1 (Continued)

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
14	Rat	(G)	1 d	Renal	2450			Hewitt et al. 1983
15	Mouse	(G)	14 d 1x/d	Renal		37[b] (minimal histolog. changes)		Condie et al. 1983
16	Mouse	(G)	14 d 1x/d	Hepatic		37[b] (minimal histolog. changes)		Condie et al. 1983
17	Mouse	(G)	14 d 1x/d	Hepatic	50	125 (incr. liver wt.)		Munson et al. 1982
18	Mouse	(G)	14 d 1x/d	Renal	250	500 (reddened medullae)		NTP 1985
19	Mouse	(G)	14 d 1x/d	Gastro	60	125 (stomach nodules)		NTP 1985
20	Mouse	(G)	14 d 1x/d	Hepatic	250	500 (mottled liver)		NTP 1985
Immunological								
21	Mouse	(G)	14 d 1x/d		50		125 (decr. immunity)	Munson et al. 1982
Neurological								
22	Rat	(G)	14 d 1x/d		250	500 (lethargy, ataxia)		NTP 1985
23	Rat	(G)	1 d		160	310 (lethargy)		NTP 1985
24	Mouse	(G)	1 d				500 (sedation)	Bowman et al. 1978
25	Mouse	(G)	1 d				454 (ED50, coordination)	Balster and Borzelleca 1982
26	Mouse	(G)	14 d 1x/d		250	500 (CNS depression)		NTP 1985

TABLE 2-1 (Continued)

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	Serious (mg/kg/day)	Reference
Developmental								
27	Rat	(G)	9 d Gd 6-15		200			Ruddick et al. 1983
INTERMEDIATE EXPOSURE								
Death								
28	Rat	(G)	13 wk 5d/wk		125		250 (18 of 20 died)	NTP 1985
29	Rat	(W)	28 d		60			Chu et al. 1982a
30	Mouse	(G)	13 wk 5d/wk		250			NTP 1985
Systemic								
31	Rat	(W)	28 d	Renal	60			Chu et al. 1982a
32	Rat	(G)	13 wk 5d/wk	Renal	125		250 (nephropathy)	NTP 1985
33	Rat	(W)	28 d	Hepatic	60			Chu et al. 1982a
34	Rat	(G)	13 wk 5d/wk	Hepatic		30 (vacuolization)	250 (necrosis)	NTP 1985
35	Mouse	(G)	13 wk 5d/wk	Renal	125	250[c] (nephrosis)		NTP 1985
36	Mouse	(G)	13 wk 5d/wk	Hepatic	125	250[c] (vacuolar changes)		NTP 1985
Neurological								
37	Rat	(G)	13 wk 5d/wk		250			NTP 1985

TABLE 2-1 (Continued)

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
38	Mouse	(G)	13 wk 5d/wk		250			NTP 1985
39	Mouse	(G)	30-90 d 1x/d		100	400 (decr. operant behavior)		Balster and Borzelleca 1982
Developmental								
40	Mouse	(W)	2 generations (continuous)		685			Borzelleca and Carchman 1982
Reproductive								
41	Mouse	(W)	2 generations (continuous)		170		685 (decr. fertility)	Borzelleca and Carchman 1982
42	Mouse	(G)	13 wk 5d/wk		250			NTP 1985
CHRONIC EXPOSURE								
Systemic								
43	Rat	(G)	2 yr 5d/wk	Renal		40[d] (nephrosis)		NTP 1985
44	Rat	(F)	1-2 yr	Hepatic		44[e] (yellow, enlarged liver)		Tobe et al. 1982
45	Rat	(G)	2 yr 5d/wk	Hepatic		40[d] (fatty change)		NTP 1985
46	Mouse	(G)	105 wk 5d/wk	Renal			50 (nephrosis)	NTP 1985
47	Mouse	(G)	105 wk 5d/wk	Hepatic			50 (necrosis)	NTP 1985

TABLE 2-1 (Continued)

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
Neurological								
48	Rat	(G)	2 yr 5d/wk		80			NTP 1985
49	Mouse	(G)	105 wk 5d/wk		100			NTP 1985
Reproductive								
50	Rat	(G)	2 yr 5d/wk		80			NTP 1985
51	Mouse	(G)	105 wk 5d/wk		100			NTP 1985
Cancer								
52	Mouse	(G)	105 wk 5d/wk				100 (liver tumors)	NTP 1985

[a]Converted to an equivalent concentration of 2,600,000 ppb in water for presentation in Table 1-4.
[b]Used to derive acute oral MRL; dose divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). Dose of 37 mg/kg/day also converted to an equivalent concentration of 190,000 ppb in water for presentation in Table 1-4. MRL of 0.04 mg/kg/day converted to an equivalent concentration of 1,300 ppb in water for presentation in Table 1-3.
[c]Converted to an equivalent concentration of 1,300,000 ppb in water for presentation in Table 1-4.
[d]Used to calculate chronic oral MRL: Dose adjusted for intermittant exposure, and divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability). Dose of 40 mg/kg/day also converted to an equivalent concentration of 290,000 ppb in water for presentation in Table 1-4. MRL of 0.03 mg/kg/day converted to an equivalent concentration of 1,000 ppb in water for presentation in Table 1-3.
[e]Converted to an equivalent concentration of 880,000 ppb in food for presentation in Table 1-4.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; mg = milligram; kg = kilograms; G = gavage; d = day; LD_{50} = lethal dose, 50% mortality; et al. = and others; x = time; incr. = increased; ser. = serum; enz = enzymes; histolog. = histological; wt. = weight; decr. = decreased; ED_{50} = dose at which 50% of the maximal effect occurs; CNS = central nervous system; Gd = gestation day; wk = week; W = water; yr = year; F = feed.

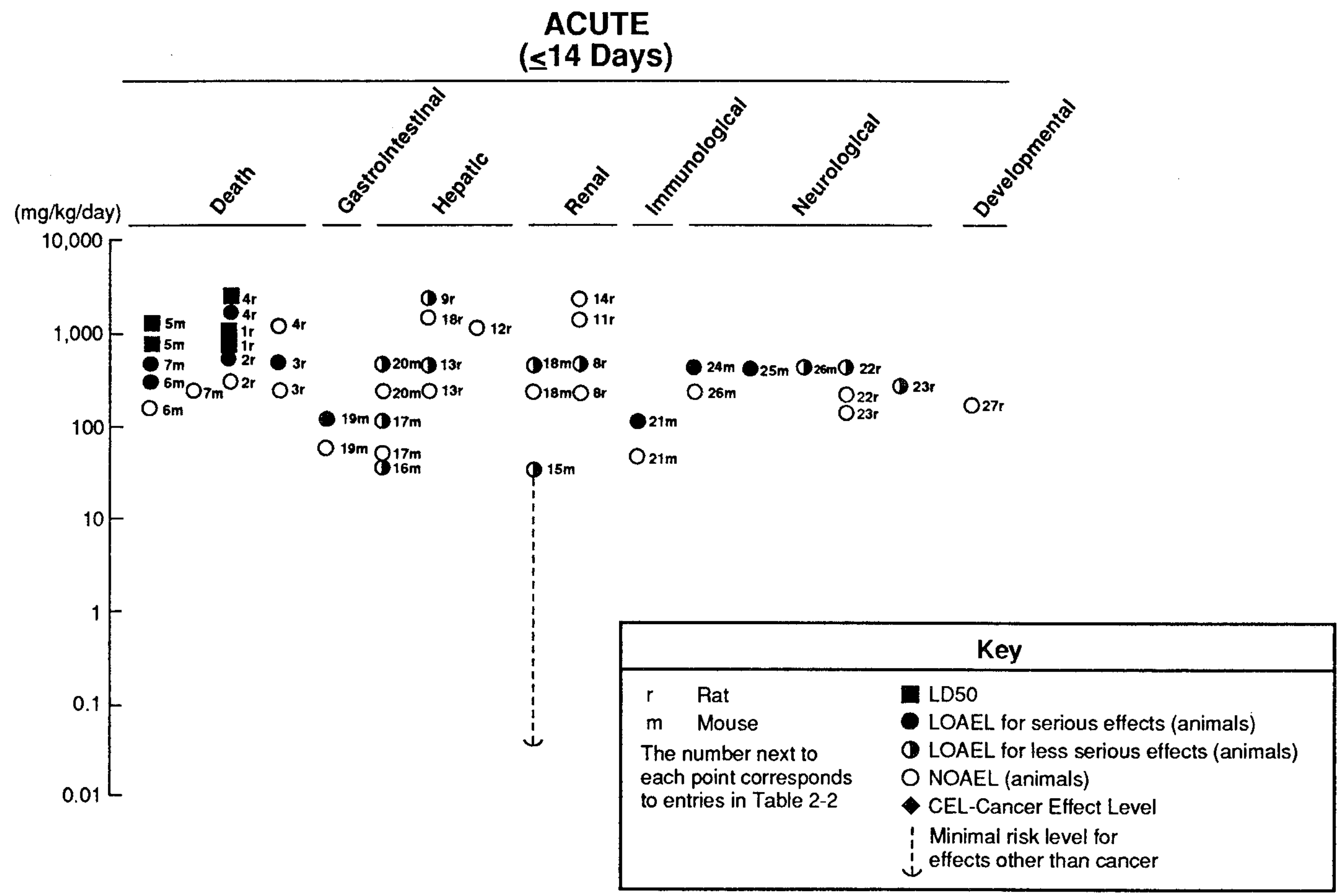

FIGURE 2-1. Levels of Significant Exposure to Chlorodibromomethane – Oral

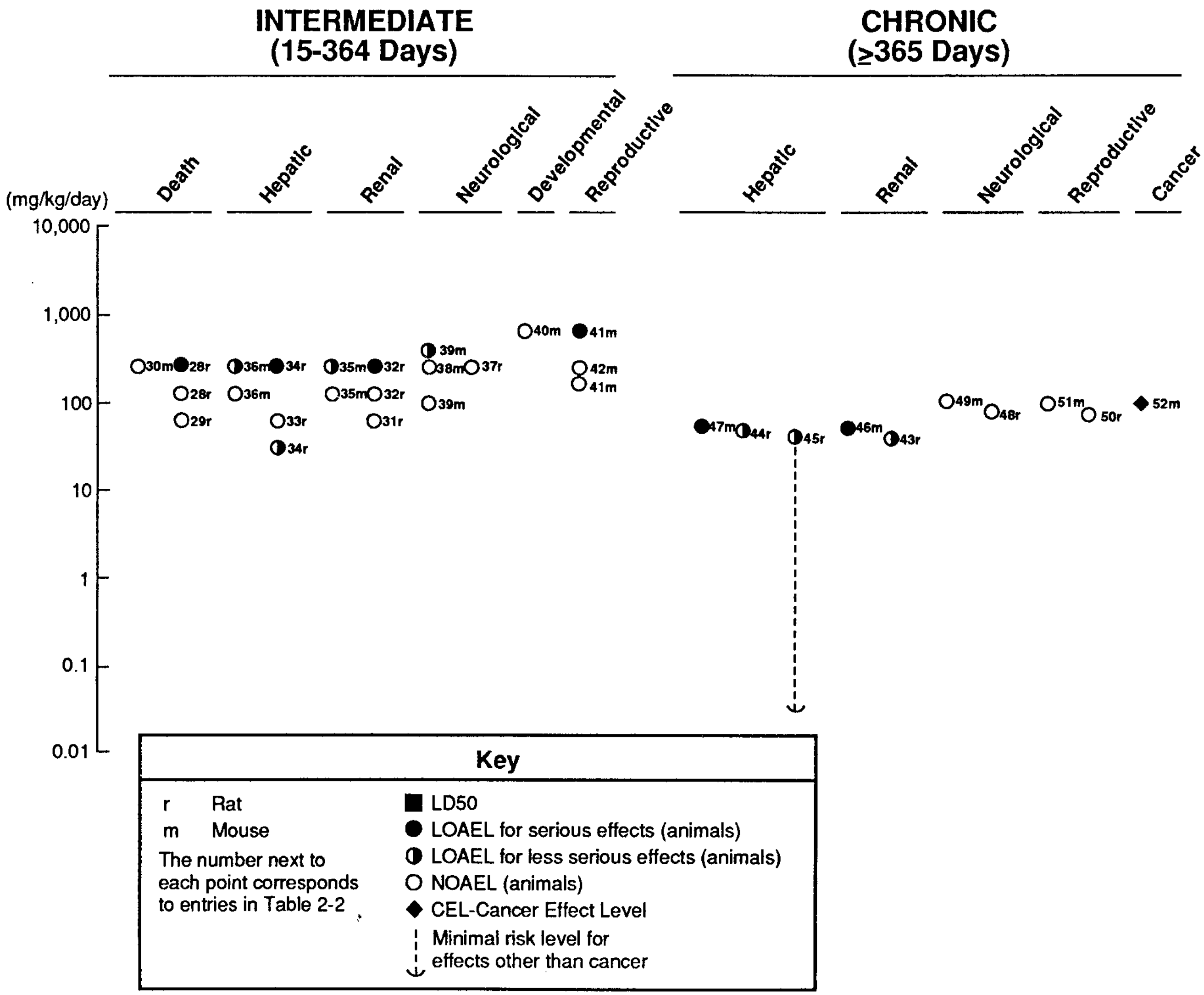

FIGURE 2-1 (Continued)

TABLE 2-2. Levels of Significant Exposure to Bromoform - Oral

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
ACUTE EXPOSURE								
Death								
1	Human		1 d				270	Dwelle 1903
2	Rat	(G)	1 d				1388 (LD50 males) 1147 (LD50 females)	Chu et al. 1982a
3	Rat	(G)	1 d		500		1000 (6 of 10 died)	NTP 1988
4	Rat	(G)	14 d 1x/d		200		400[a] (1 of 10 died)	NTP 1988
5	Mouse	(G)	1 d				1400 (LD50 males) 1550 (LD50 females)	Bowman et al. 1978
6	Mouse	(G)	1 d		250		500 (1 of 10 died)	NTP 1988
7	Mouse	(G)	14 d 1x/d		289			Condie et al. 1983
8	Mouse	(G)	14 d 1x/d		400			NTP 1988
9	Mouse	(G)	14 d 1x/d		250			Munson et al. 1982
Systemic								
10	Rat	(G)	1 d	Hepatic	765	1071 (histolog. changes)		Chu et al. 1982a
11	Rat	(G)	1 d	Hepatic	1440			Plaa and Hewitt 1982a
12	Rat	(G)	1 d	Renal	1500			Chu et al. 1982a
13	Rat	(G)	1 d	Hepatic	29			Klingensmith and Mehendale 1981
14	Rat	(G)	1 d	Hepatic		1000 (altered enzymes)		Moody and Smuckler 1986

TABLE 2-2 (Continued)

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
15	Mouse	(G)	14 d 1x/d	Renal	72	145 (minimal histolog. changes)		Condie et al. 1983
16	Mouse	(G)	14 d 1x/d	Gastro	200		400 (stom. nodules)	NTP 1988
17	Mouse	(G)	14 d 1x/d	Hepatic		145 (histolog. changes)		Condie et al. 1983
18	Mouse	(G)	14 d 1x/d	Hepatic	50	125[b] (incr. liver wt.)		Munson et al. 1982
Immunological								
19	Mouse	(G)	14 d 1x/d		125		250 (decr. immunity)	Munson et al. 1982
Neurological								
20	Human		1 d			60[c] (sedation)	270 (severe CNS depr.)	Dwelle 1903
21	Mouse	(G)	14 d 1x/d			600 (ataxia)		NTP 1988
22	Mouse	(G)	1 d				431 (ED50, coordination)	Balster and Borzelleca 1982
23	Mouse	(G)	1 d				1000 (sedation)	Bowman et al. 1978
Developmental								
24	Rat	(G)	9 d Gd 6-15		50		100 (skeletal anom.)	Ruddick et al. 1983
INTERMEDIATE EXPOSURE								
Death								
25	Rat	(G)	13 wk 5d/wk		200			NTP 1988
26	Rat	(W)	28 d		70			Chu et al. 1982a
27	Mouse	(G)	13 wk 5d/wk		400			NTP 1988

TABLE 2-2 (Continued)

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
Systemic								
28	Rat	(W)	28 d	Renal	70			Chu et al. 1982a
29	Rat	(W)	28 d	Hepatic	70			Chu et al. 1982a
30	Rat	(G)	13 wk 5d/wk	Hepatic		50 (vacuolization)		NTP 1988
31	Mouse	(G)	13 wk 5d/wk	Hepatic	100	200 (vacuolization)		NTP 1988
Neurological								
32	Rat	(G)	13 wk 5d/wk			100 (lethargy)		NTP 1988
33	Mouse	(G)	13 wk 5d/wk		400			NTP 1988
34	Mouse	(G)	30-90 d 1x/d		9.2	100 (decr. operant behavior)		Balster and Borzelleca 1982
Reproductive								
35	Mouse	(G)	105 d 1x/d		200			NTP 1989
CHRONIC EXPOSURE								
Systemic								
36	Rat	(G)	103 wk 5d/wk	Hepatic		100[d] (inflammation, fatty change)		NTP 1988
37	Rat	(G)	103 wk 5d/wk	Gastro			100 (ulcer)	NTP 1988
38	Rat	(F)	1-2 yr	Hepatic	20[e]	80[f] (yellow, enlarged liver)		Tobe et al. 1982
39	Mouse	(G)	103 wk 5d/wk	Gastro		50 (hyperplasia)		NTP 1988

TABLE 2-2 (Continued)

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
40	Mouse	(G)	103 wk 5d/wk	Hepatic		100[d] (fatty change)		NTP 1988
Neurological								
41	Rat	(G)	103 wk 5d/wk		200			NTP 1988
42	Mouse	(G)	103 wk 5d/wk		200			NTP 1988
Reproductive								
43	Rat	(G)	103 wk 5d/wk		200			NTP 1988
44	Mouse	(G)	103 wk 5d/wk		200			NTP 1988
Cancer								
45	Rat	(G)	103 wk 5d/wk				200 (intestinal tumors)	NTP 1988

[a]Converted to an equivalent concentration of 2,900,000 ppb in water for presentation in Table 1-4.
[b]Converted to an equivalent concentration of 660,000 ppb in water for presentation in Table 1-4.
[c]Used to derive acute oral MRL; dose divided by an uncertainty factor of 100 (10 for use of a LOAEL, and 10 for human variability). Dose of 60 mg/kg/day and MRL of 0.6 mg/kg/day converted to an equivalent concentrations of 2,100,000 and 21,000 ppb in water for presentation in Table 1-3.
[d]Converted to an equivalent concentration of 530,000 ppb in water for presentation in Table 1-4.
[e]Used to derive chronic MRL; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability). MRL of 0.2 mg/kg/day converted to an equivalent concentration of 6,900 ppb in water for presentation in Table 1-3.
[f]Converted to an equivalent concentration of 1,600,000 ppb in food for presentation in Table 1-4.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; mg = milligram; kg = kilogram; d = day; G = Gavage; LD_{50} = lethal dose, 50% mortality; x = time; histolog. = histological; Gastro = gastrointestinal; F = feed; stom. = stomach; incr. = increased; wt. = weight; decr. = decreased; CNS = central nervous system; depr. = depression; ED_{50} = dose at which 50% of the maximal effect occurs; Gd = gestation day; anom = anomaly; W = water; wk = week; yr = year.

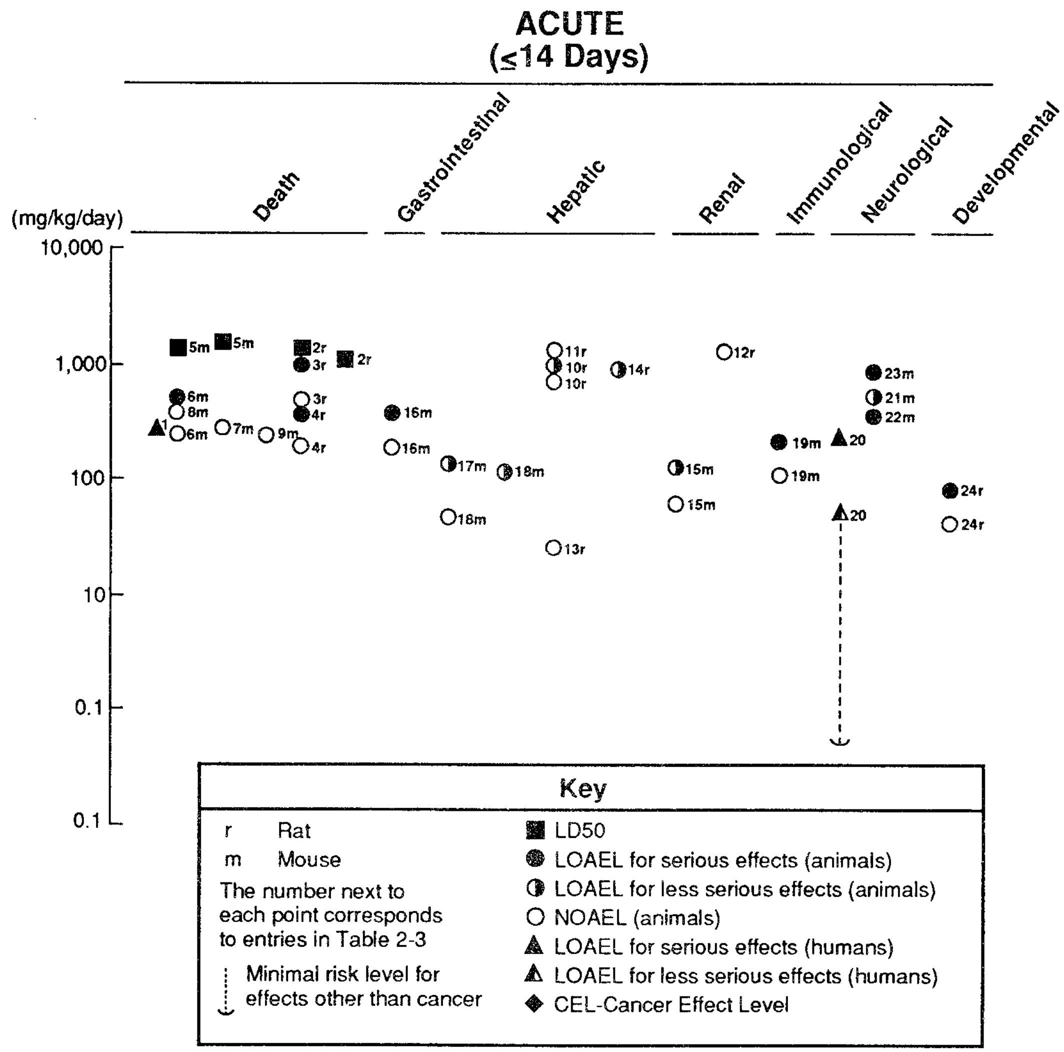

FIGURE 2-2. Levels of Significant Exposure to Bromoform – Oral

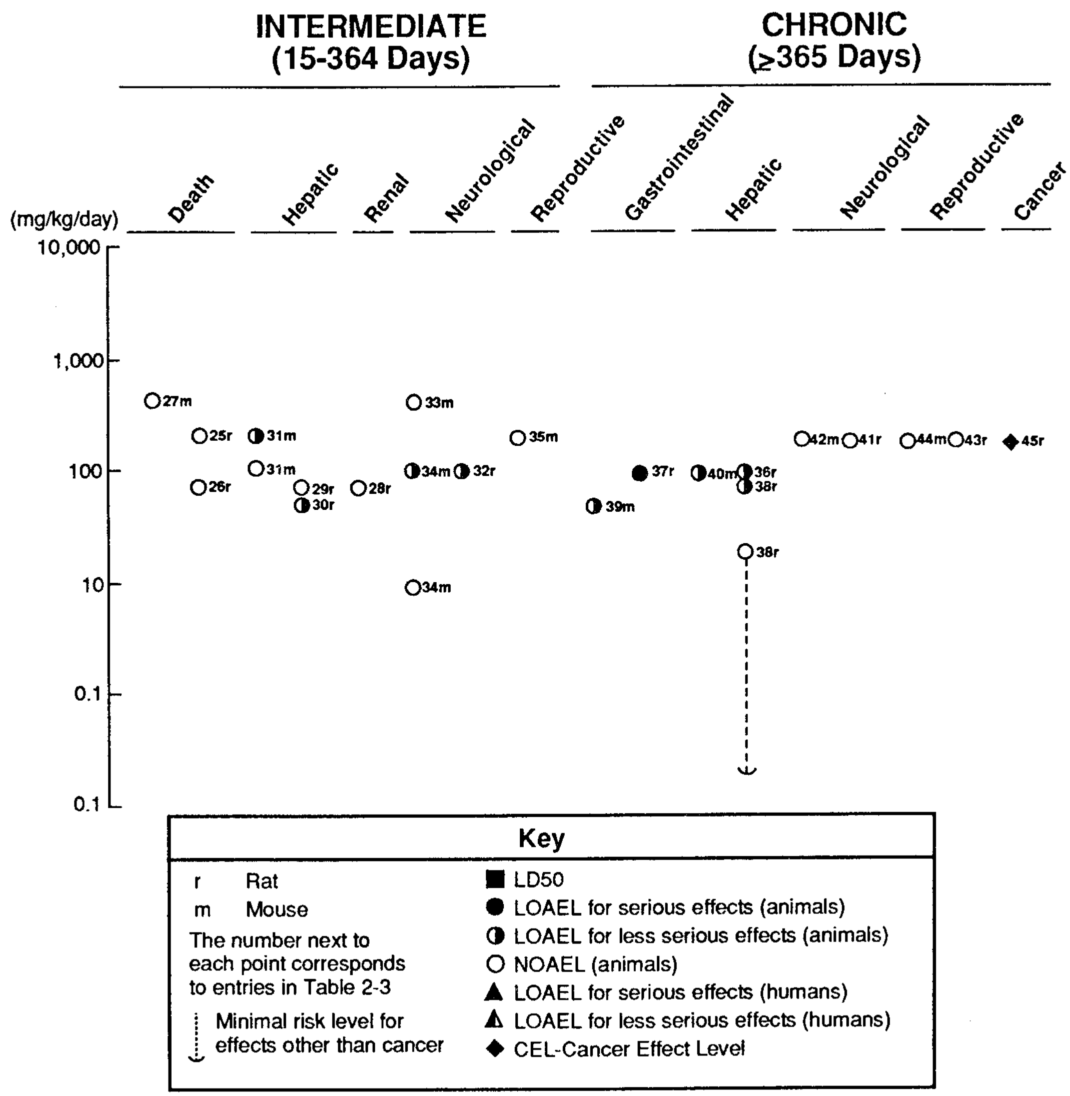

FIGURE 2-2 (Continued)

by observations in long-term studies, where deaths in rats dosed with 250 mg/kg/day of chlorodibromomethane did not occur until exposure had continued for 8 to 10 weeks (NTP 1985).

2.2.2.2 Systemic Effects

Respiratory Effects. Histological examination of larynx, trachea, lungs, and bronchi of rats and mice exposed to chlorodibromomethane (80 to 100 mg/kg/day) or bromoform (100 to 200 mg/kg/day) by gavage for up to two years revealed no evidence of adverse effects, except for an increased incidence of chronic inflammation of the lungs in male rats exposed to bromoform (NTP 1985, 1988). This inflammation was similar in appearance to that caused by a sialodacryoadenitis (SDA) virus infection, and antibodies to rat SDA virus were detected in study animals. Thus, the inflammation observed was probably secondary to the infection and was not a direct result of bromoform. However, the absence of symptoms in control animals suggested that bromoform-treated rats may have been more susceptible to reinfection by the virus or slower to recover (NTP 1988).

Cardiovascular Effects. Histological examination of rats and mice exposed to chlorodibromomethane or bromoform by gavage for up to two years revealed no evidence of adverse effects upon the heart (NTP 1985, 1988). While this indicates that cardiac tissue is not directly injured by these chemicals, indirect effects on cardiovascular functions might occur as a consequence of the central nervous system depressant activity of these compounds (see Section 2.2.2.3). However, this has not been studied.

Gastrointestinal Effects. Effects of chlorodibromomethane and bromoform on the gastrointestinal tract have not been widely studied, but histological examinations of stomach and intestine from rats and mice exposed to these chemicals by gavage have been performed by NTP (NTP 1985, 1988). In mice, raised nodules were observed in the stomach following 14 days exposure to 125 mg/kg/day of chlorodibromomethane or 400 mg/kg/day of bromoform. These nodules were not observed in rats exposed to chlorodibromomethane for 14 days, and were not observed in either rats or mice exposed to doses of 80 to 100 mg/kg/day of chlorodibromomethane or 100 to 200 mg/kg/day of bromoform for 90 days to 2 years. The biological significance of these nodules is not immediately apparent, but it is likely that they are a response to a direct irritant effect of the chemicals on the gastric mucosa.

Another gastrointestinal effect of potential concern is the occurrence of ulcers in the forestomach of male rats exposed to 100 or 200 mg/kg/day of bromoform for two years (NTP 1988). This effect was not observed in female rats or in mice exposed to bromoform, although mice exposed to bromoform displayed a dose-dependent hyperplasia of the glandular stomach.

While these observations clearly indicate that the stomach may be affected by chlorodibromomethane and bromoform, it is possible that the exposure regimen (bolus dosing, by gavage, in oil) leads to irritant effects in the stomach that might not occur if exposure were continuous at lower concentrations in food or drinking water. However, this has not been investigated.

Hematological Effects. Several studies (Chu et al. 1982a, 1982b; Munson et al. 1982; Tobe et al. 1982) have investigated the hematological effects of oral exposure of rats and mice to chlorodibromomethane and bromoform. With the exceptions of some minor fluctuations in lymphocyte count following exposure to bromoform (Chu et al. 1982a, 1982b), none of these studies detected any significant effects of chlorodibromomethane or bromoform on hemoglobin, hematocrit, red blood cells, or white blood cells.

Musculoskeletal Effects. None of the available studies on the oral toxicity of chlorodibromomethane or bromoform have reported effects on the musculoskeletal system. However, detailed electrophysiologic or histopathologic studies on these tissues have not been performed.

Hepatic Effects. Nearly all studies of chlorodibromomethane and bromoform toxicity in rats and mice indicate that the liver is a target tissue for these chemicals. However, hepatic effects are usually not severe, being characterized most often by increased vacuolization, fat accumulation, increased liver weight, and altered serum enzyme levels. Small changes of this sort have been detected in some experiments following exposure for 2 to 13 weeks at doses as low as 30 to 50 mg/kg/day (Condie et al. 1983; NTP 1985; Tobe et al. 1982), and hepatic effects are frequently reported after doses of 50 to 500 mg/kg/day (Condie et al. 1983; Munson et al. 1982; NTP 1985, 1988). Occasionally centrilobular necrosis may develop (NTP 1985), but this is rarely extensive.

Chlorodibromomethane and bromoform appear to be of approximately similar hepatotoxicity (Condie et al. 1983; NTP 1985, 1988). Males tend to be more sensitive to chlorodibromomethane and bromoform than females, and mice tend to be more sensitive than rats (NTP 1985, 1988), but these differences also are not large. The basis for the variability between chemicals, species and sexes is probably related to differences in the metabolism of these compounds (see Section 2.6), but this has not been rigorously established.

Based on a LOAEL of 40 mg/kg/day for hepatic injury from chlorodibromomethane (NTP 1985), a chronic oral MRL of 0.03 mg/kg/day was calculated as described in footnote d in Table 2-l. For bromoform, a NOAEL value of 20 mg/kg/day (Tobe et al. 1982) was used to calculate a chronic oral MRL of 0.2 mg/kg/day, as described in footnote c in Table 2-2.

Renal Effects. Histological studies performed by NTP (1985) indicate that oral exposure to chlorodibromomethane can cause kidney injury in both rats and mice. The medullae appear to be reddened in both males and females after a single oral dose of 500 mg/kg, but this dose was so high that 7 of 10 animals died. Of greater toxicological concern are effects on the nephron that develop after intermediate or chronic exposure to doses of 50 to 250 mg/kg/day (NTP 1985). These effects are usually much more apparent in males than females, and are characterized by tubular degeneration and mineralization leading to nephrosis (NTP 1985). These histological findings of nephrotoxicity are supported by the kidney function studies of Condie et al. (1983), who found that ingestion of chlorodibromomethane (37 to 147 mg/kg/day) for 2 weeks by male mice tended to impair uptake of para-amino hippuric acid (PAH) in renal slices prepared from exposed animals. Based on a value of 37 mg/kg/day, an acute oral MRL of 0.04 mg/kg/day was calculated for chlorodibromomethane, as described in footnote b in Table 2-l.

Bromoform also has nephrotoxic potential. Condie et al. (1983) noted minimal to slight nephrosis and mesangial hypertrophy in male mice exposed to repeated oral doses of 145 to 289 mg/kg/day of bromoform. However, in contrast to the findings for chlorodibromomethane (see above), no significant histopathological effects were detected by NTP (1988) in rats or mice exposed to doses up to 200 mg/kg of bromoform for two years. This suggests that bromoform may be somewhat less nephrotoxic than chlorodibromomethane, but the data are too limited to draw a firm conclusion. The basis for the difference in nephrotoxicity between chlorodibromomethane and bromoform has not been thoroughly studied, but is possibly related to differences in the renal metabolism of these two compounds.

Dermal/Ocular Effects. Histological studies of tissues from rats and mice exposed to chlorodibromomethane or bromoform by gavage for up to two years revealed no treatment-related effects on skin or eyes (NTP 1985, 1988).

2.2.2.3 Immunological Effects

Only one study (Munson et al. 1982) has formally investigated the effect of chlorodibromomethane and bromoform ingestion on the immune system. Exposure of mice to doses of 125 or 250 mg/kg/day of chlorodibromomethane for 14 days lead to decreases in several indices of humoral and cell-mediated immunity in both males and females. Similar effects were observed in male mice exposed to 250 mg/kg of bromoform, but no effects were noted in females. These observations indicate that several cell-types of the immune system are affected by chlorodibromomethane and bromoform, but the data do not reveal whether these changes are accompanied by a significant decrease in immune system function (e.g., decreased resistance to infectious disease). In this regard, it should be recalled that male rats exposed to bromoform for two years appeared to have decreased resistance to a common viral infection (NTP 1988), suggesting (but not proving) that bromoform may have led to functional impairment of the immune system in these animals.

2.2.2.4 Neurological Effects

Both chlorodibromomethane and bromoform, like other volatile halogenated hydrocarbons, can lead to marked central nervous system depression. Because of this property, bromoform was used as a sedative in the early part of this century. Doses of 1 to 2 drops (probably about 15 to 20 mg/kg) given 3 to 6 times per day usually produced sedation (the ability to sleep) in children with whooping cough (Burton-Fanning 1901; Dwelle 1903). This dose (probably averaging around 60 mg/kg/day) has been used to calculate an acute oral MEL for bromoform of 0.6 mg/kg/day, as described in footnote c of Table 2-2. In mild cases of accidental overdose, clinical signs included rapid breathing, constricted pupils, and tremors; more severe cases of overdose were accompanied by a drunken-like stupor, cyanosis, shallow breathing, and erratic heart rate (Benson 1907; Kobert 1906). Doses producing these effects could only be estimated, but most were probably in the range of 20 to 40 drops (corresponding to doses of about 150 to 300 mg/kg).

Very similar effects on the nervous system are observed in animals exposed to bromoform or chlorodibromomethane. Acute signs such as labored breathing, ataxia, and sedation are generally observed only after doses of 300 mg/kg or above (Balster and Borzelleca 1982; Bowman et al. 1978; NTP 1985, 1988). These effects appear quickly (within one hour) and persist for a number of hours. Following repeated exposure to lower doses, lethargy is the main effect (NTP 1988). It is not known whether high doses of chlorodibromomethane or bromoform lead to any histopathological changes in the brain, but intermediate (13 week) or chronic (2 year) exposure of rats and mice to subanesthetic doses produced no histological changes in the brain (NTP 1985, 1988).

Balster and Borzelleca (1982) employed a series of behavioral tests to investigate the neurological effects of chlorodibromomethane and bromoform in mice. Doses of 9 or 10 mg/kg/day for 90 days did not have any significant effects on performance in tests of strength, activity, or coordination. Exposure to higher doses (100 or 400 mg/kg/day) for 30 to 90 days had no effect on passive avoidance learning, but did cause a transient decrease in response rate in a test of operant behavior. It should be noted that a number of animals receiving the high dose died during the study.

These studies suggest that the depressant effects of chlorodibromomethane and bromoform on the nervous system are probably not accompanied by any lasting behavioral or histological alterations.

2.2.2.5 Developmental Effects

The developmental effects of oral exposure to chlorodibromomethane and bromoform have not been extensively investigated, but limited data suggest these chemicals have relatively low toxicity on the developing fetus. Ruddick et al. (1983) dosed pregnant rats with up to 200 mg/kg/day of chlorodibromomethane or bromoform during gestation. An increased incidence of minor skeletal anomalies was noted at doses of 100 and 200 mg/kg/day of bromoform, but no other significant fetotoxicity or teratogenicity was detected. Borzelleca and Carchman (1982) exposed mice to 685 mg/kg/day of chlorodibromomethane in drinking water for several generations and detected no significant effect on the incidence of gross, skeletal, or soft-tissue anomalies.

2.2.2.6 Reproductive Effects

Chronic exposure of rats and mice to chlorodibromomethane (80 to 100 mg/kg/day) or bromoform (100 to 200 mg/kg/day) resulted in no detectable histological effects in reproductive tissues of males (testes, prostate, and seminal vesicles) or females (ovaries, uterus, and mammary gland) (NTP 1985, 1988). In a detailed study of the effects of bromoform on reproduction and fertility in male and female mice, doses up to 200 mg/kg/day had no significant effect (NTP 1989).

In contrast to these negative findings, female mice exposed to chlorodibromomethane in drinking water at a high dose (685 mg/kg/day) experienced a marked reduction in fertility, with significant decreases in litter size, gestational survival, postnatal survival, and postnatal body weight (Borzelleca and Carchman 1982). These affects may have been due to marked maternal toxicity, as evidenced by decreased weight gain, enlarged and discolored livers, and decreased survival. Exposure to lower doses (17 or 170 mg/kg/day) resulted in occasional decreases in one or more of the reproductive parameters monitored, but the effects were not large and were not clearly dose-related. These data are not sufficient to draw firm conclusions about the effects of chlorodibromomethane on reproduction, but it appears that reproductive tissues and functions are not markedly impaired at doses that do not cause frank maternal toxicity.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects of chlorodibromomethane or bromoform in humans exposed by the oral route. Morimoto and Koizumi (1983) found an increased frequency of sister chromatid exchange in bone marrow cells from mice given oral doses of 25 to 250 mg/kg/day of chlorodibromomethane or bromoform for four days. Other in vivo and in vitro studies on the genotoxicity of chlorodibromomethane and bromoform are presented and discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding carcinogenic effects in humans following oral exposure to chlorodibromomethane or bromoform. There are a number of epidemiological studies that indicate there may be an association between chronic ingestion of chlorinated drinking water (which typically contains chlorodibromomethane and bromoform) and increased risk of rectal, bladder, or colon cancer in humans (Cantor et al. 1987; Crump 1983; Kanarek and Young 1982; Marienfeld et al. 1986), but these studies cannot provide information on whether any effects observed are due to chlorodibromomethane, bromoform, or to one or more of the hundreds of other byproducts that are also present in chlorinated water.

Chronic oral studies in animals indicate that both chlorodibromomethane and bromoform have carcinogenic effects. The key findings are summarized in Table 2-3. Chronic exposure to chlorodibromomethane resulted in an increased incidence of liver tumors (adenomas or carcinomas) in mice (but not in rats) (NTP 1985), and bromoform caused an increased frequency of neoplasms of the large intestine (adenomatous polyps or adenocarcinomas) in rats (but not in mice) (NTP 1988). Even though the absolute incidence of intestinal neoplasms in bromoform-treated rats was relatively low, the data constitute clear evidence for the tumorigenicity of bromoform, since these lesions are rare in control animals. For chlorodibromomethane, the evidence is more limited, but the data are still indicative of carcinogenic potential.

The mechanism of carcinogenicity of chlorodibromomethane, bromoform and other related trihalomethanes (THMs) such as bromodichloromethane and chloroform is not known, but might be related to the metabolic generation of a reactive dihalocarbonyl intermediate (see Section 2.3). If so, the differences noted between tissues, sexes, and species regarding the carcinogenic effect of any given THM could be related to differences in the rate of generation of this intermediate. Likewise, differences in potency and specificity between different THMs could be related not only to the relative rate of metabolism to the dihalocarbonyl, but also to the reactivity of the resulting

TABLE 2-3. Summary of Lifetime Carcinogenicity Bioassay Findings

Chemical (Reference)	Test Species	Sex	Tissues with Increased Tumors	Tumor Type(s)	Statistical Significance	Level of Evidence Category[a]
Chlorodibromomethane (NTP 1985)	Rat	M	None	--	--	No evidence
		F	None	--	--	No evidence
	Mouse	M	Liver	Adenoma	NS	
				Carcinoma	0.030	Equivical evidence
				Adenoma or Carcinoma	0.065	
		F	Liver	Adenoma	0.016	
				Carcinoma	0.258	Some evidence
				Adenoma or Carcinoma	0.004	
Bromoform (NTP 1988)	Rat	M	Large Intestine	Adenomatous polyps	NS	
				Adenocarcinoma	NS	Some evidence
				Polyps or Adenocarcinoma	0.028	
		F	Large Intestine	Adenomatous polyps	0.015	
				Adenocarcinoma	NS	Clear evidence
				Polyps or adenocarcinoma	0.004	
	Mouse	M	None	--	--	No evidence
		F	None	--	--	No evidence

[a]These are specific level-of-evidence categories assigned by NTP. Refer to the source documents (NTP 1985 or NTP 1988) for a full description of the meaning of these categories.

M = male; F = female; NS = Not statistically significant (P>0.05 by life-table test or logistic regression test).

dihalocarbonyl. The apparent carcinogenic potency in the liver appears to be inversely related to the chemical reactivity of the dihalocarbonyl (NTP 1988). That is, THMs such as chloroform and bromodichloromethane which generate dichlorocarbonyl (the least chemically reactive) are more potent than THMs such as iodoform or bromoform which generate the more reactive diiodocarbonyl or dibromocarbonyl groups. This may be because the most highly reactive dihalocarbonyls are more readily destroyed by reaction with glutathione, while the less reactive species are more likely to diffuse into the nucleus and react with DNA before they are destroyed (NTP 1988).

2.2.3 Dermal Exposure

No studies were located regarding the following health effects in humans or animals after dermal exposure to chlorodibromomethane or bromoform.

2.2.3.1 Death

2.2.3.2 Systemic Effects

2.2.3.3 Immunological Effects

2.2.3.4 Neurological Effects

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

2.2.3.8 Cancer

2.3 TOXICOKINETICS

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

No studies were located regarding the rate or the extent of chlorodibromomethane or bromoform absorption in humans or animals following inhalation exposure. Based on the physical-chemical properties of these compounds, and by analogy with other related halomethanes such as chloroform (ATSDR 1989a), it is expected that bot chlorodibromomethane and bromoform would be well-absorbed across the lung. The occurrence of systemic and neurological effects following inhalation exposure of animals to bromoform (see Section 2.2.1) supports this view.

2.3.1.2 Oral Exposure

Only one study was located which provides quantitative data on gastrointestinal absorption of chlorodibromomethane and bromoform. Mink et al. (1986) found that 60% to 90% of single oral doses of these compounds given in corn oil to rats or mice were recovered in expired air, urine, or in internal organs. This indicates that gastrointestinal absorption was at least 60% to 90% complete. This is consistent with the ready gastrointestinal absorption observed for other halomethanes such as chloroform (ATSDR 1989a) or carbon tetrachloride (ATSDR 1989b). As noted by Withey et al. (1983), the rate of halocarbon uptake from the gastrointestinal tract may be slower when compounds are given in oil than when they are given in water.

2.3.1.3 Dermal Exposure

No studies were located regarding dermal absorption of chlorodibromomethane or bromoform in humans or animals. The dermal permeability constant for chloroform in aqueous solution has been estimated to be 0.125 cm/hr (Beech 1980). Assuming that chlorodibromomethane and bromoform have similar permeability constants, flux rates of around 10 $ng/cm^2/hr$ could occur during dermal contact with water containing 100 μg/L of these chemicals (Beech 1980).

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies were located regarding the distribution of chlorodibromomethane or bromoform in humans or animals following inhalation exposure. However, adverse effects involving several organs (liver, kidney, central nervous system) indicates distribution to these sites.

2.3.2.2 Oral Exposure

The distribution of chlorodibronomethane and bromoform in tissues following oral exposure has not been thoroughly investigated. Analysis of bromoform levels in the organs of a child who died after an accidental overdose revealed concentrations of 10 to 40 mg bromoform per kg tissue in intestine, liver, kidney, and brain, with somewhat higher levels in lung (90 mg/kg) and stomach (130 mg/kg) (Roth 1904, as cited in von Oettingen 1955). This suggests that bromoform is distributed fairly evenly from the stomach to other tissues.

In animals, Mink et al. (1986) found that only about 1 to 2% of a single oral dose of ^{14}C-labeled chlorodibromomethane or bromoform was retained in the soft tissues of rats eight hours after dosing. The tissues which contained measurable amounts of the radiolabel were the brain, kidney, liver, lungs, muscle, pancreas, stomach (excluding contents), thymus, and urinary bladder. The relative amount of radiolabel in each tissue was not mentioned. Similar results were noted in mice, except that blood also contained a significant fraction of the total dose (10% in the case of bromoform). The chemical form of the material in the tissues (parent, metabolite, or adduct) was not reported. The form in blood also was not determined, but studies by Anders et al. (1978) suggest that some or all may have been carbon monoxide bound to hemoglobin (see Section 2.3.3).

2.3.2.3 Dermal Exposure

No studies were located regarding the distribution of chlorodibromomethane or bromoform in humans or animals following dermal exposure.

2.3.3 Metabolism

The metabolism of chlorodibromomethane, bromoform, and other THMs has been investigated by Anders and colleagues (Ahmed et al. 1977; Anders et al. 1978; Stevens and Anders 1979, 1981). The main reactions, which are not believed to be route-dependent, are shown in Figure 2-3.

The first step in the metabolism of THMs is oxidation by the cytochrome P-450 mixed function oxidase system of liver. This has been demonstrated in vitro using isolated rat liver microsomes (Ahmed et al. 1977), and in vivo, where the rate of metabolism is increased by cytochrome P-450 inducers (phenobarbital) and decreased by cytochrome P-450 inhibitors (SKF-525A) (Anders et al. 1978). The product of this reaction is presumed to be trihalomethanol, which then decomposes by loss of hydrogen and halide ions to yield the dihalocarbonyl. Although this intermediate has not been isolated, its formation has been inferred by detection of 2-oxothiazolidine-4-carboxylic acid (OZT) in an in vitro microsomal system metabolizing bromoform in the presence of cysteine (Stevens and Anders 1979). The dihalocarbonyl molecule (an analogue of phosgene) is highly reactive, and may undergo a number of reactions, including: (a) direct reaction with cellular nucleophiles to yield covalent adducts; (b) reaction with two moles of glutathione (GSH) to yield carbon monoxide and oxidized glutathione (GSSG); and (c) hydrolysis to yield CO_2.

The amount of THM metabolized by each of these pathways has not been studied in detail, but it appears that conversion to CO_2 is the main route. However, this depends on the species, the THM being

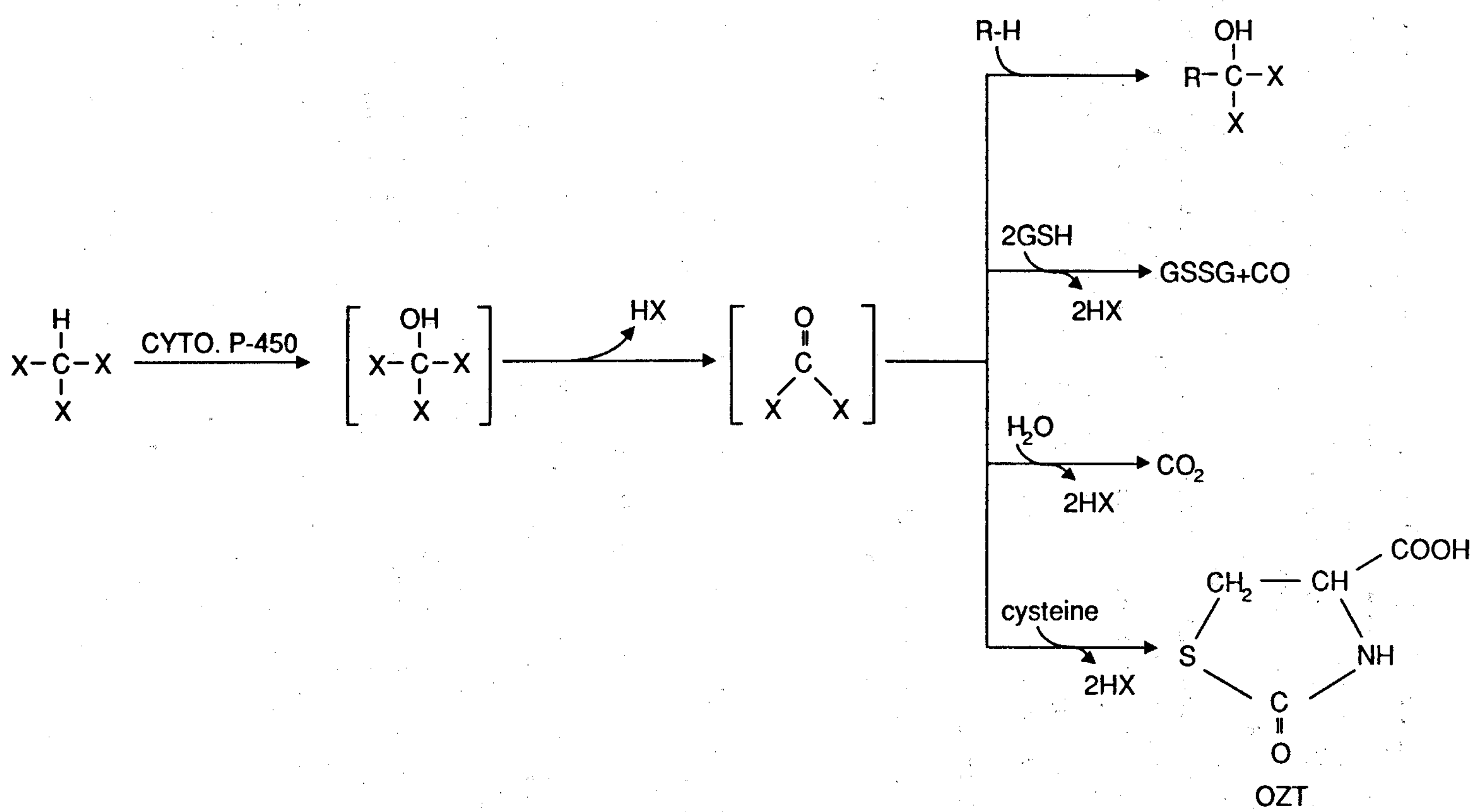

FIGURE 2-3. Proposed Pathway of Trihalomethane Metabolism in Rats*

*Adapted from Stevens and Anders 1981.

X = halogen atom (chlorine, bromine); R = cellular nucleophile (protein, nucleic acid); GSH = reduced glutathione; GSSG = oxidized glutathione; OZT = oxothiazolidine carboxylic acid.

metabolized, and metabolic conditions (cellular glutathione levels). Mink et al. (1986) found that mice oxidized 72% of an oral dose of chlorodibromomethane and 40% of an oral dose of bromoform to CO_2. In contrast, rats oxidized only 18% of chlorodibromomethane and 4% of bromoform to CO_2.

The fraction of the dose converted to carbon monoxide has not been quantified, but dramatically increased levels of carboxyhemoglobin have been reported following oral exposure of rats to bromoform (Anders et al. 1978; Stevens and Anders 1981). Mink et al. (1986) reported about 10% of a dose of bromoform was present in blood in mice; the form of the label was not investigated, but it may have been carboxyhemoglobin.

Metabolism of THMs by cytochrome P-450 can also lead to the production of highly reactive trihalomethyl free radicals, especially under hypoxic conditions (O'Brien 1988). Radical formation from bromoform has been observed both in isolated hepatocytes incubated with bromoform in vitro and in the liver of rats exposed to bromoform in vivo (Tomasi et al. 1985). Although it has not been studied, it seems likely that this pathway would also generate trihalomethyl radicals from chlorodibromomethane.

While metabolism to free radicals is a minor pathway in the sense that only a small fraction of the total dose is converted, it might be an important component of the toxic and carcinogenic mechanism of chlorodibromomethane and bromoform. Figure 2-4 shows how free radical generation can lead to an autocatalytic peroxidation of polyunsaturated fatty acids (PLJFAs) in cellular phospholipids (O'Brien 1988). Peroxidation of cellular lipids has been observed in rat kidney slices incubated with bromoform in vitro, although lipid peroxidation was not detectable in liver slices (Fraga et al. 1987). Lipid peroxidation is considered to be a likely cause of cell injury for other halogenated compounds such as Ccl_4 (ATSDR 1989b), but the significance of this pathway in the toxicity of chlorodibromomethane and bromoform remains to be determined.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

No studies were located regarding excretion of chlorodibromomethane or bromoform by humans or animals following inhalation exposure.

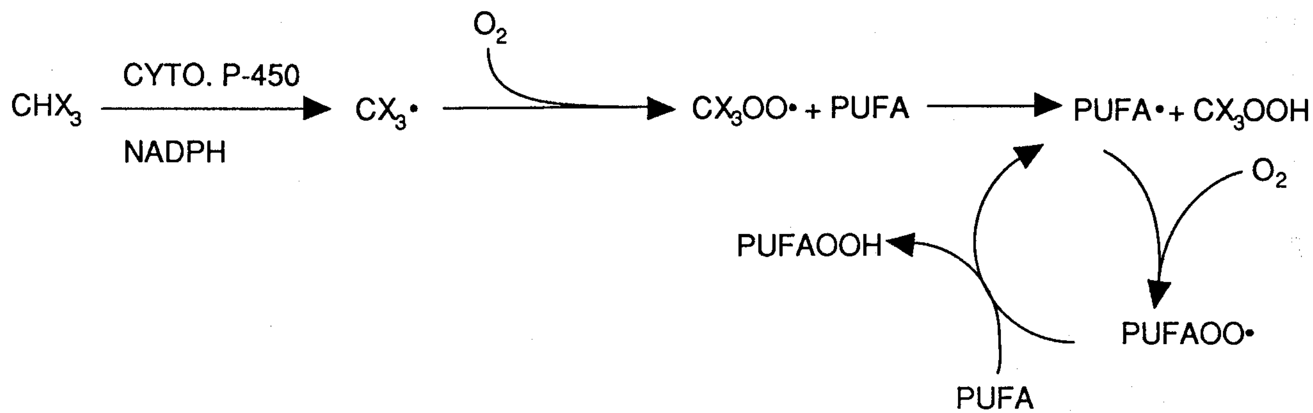

FIGURE 2-4. Proposed Pathway of Trihalomethyl-Radical-Mediated Lipid Peroxidation*

*Adapted from O'Brien 1988.

X = halogen atom (Cl, Br, I); PUFA = Polyunsaturated Fatty Acid

2.3.4.2 Oral Exposure

In rats and mice given a single oral dose of ^{14}C-labeled chlorodibromomethane or bromoform, excretion occurred primarily by exhalation of parent THM or of CO_2 (Mink et al. 1986). The total fraction of the administered dose excreted through the lungs ranged from 45% to 84%, mostly as CO_2 in mice and mostly as the parent THM in rats. Only 1% to 5% of the dose was excreted in urine (the chemical form in urine was not determined). Excretion was nearly complete within 8 hours in all cases, indicating that there is a relatively rapid clearance of the volatile species. However, significant levels (1 to 12%) of the 14C-label remained in tissues after 8 hours. The chemical form was not determined, but this might be due to stable covalent adducts formed from reactive metabolic intermediates (see Section 2.3.3).

2.3.4.3 Dermal Exposure

No studies were located regarding excretion of chlorodibromomethane or bromoform by humans or animals following dermal exposure.

2.4 RELEVANCE TO PUBLIC HEALTH

Studies in animals, combined with limited observations in humans, indicate that the principal adverse health effects associated with short-term inhalation or oral exposure to high levels of chlorodibromomethane or bromoform are central nervous system depression, liver injury, and kidney injury. Similar effects might be expected following dermal exposure to concentrated liquid chlorodibromomethane or bromoform, but this has not been studied. Because chlorodibromomethane and bromoform have very low production and use (see Chapter 4), doses needed to cause these effects are not likely to be encountered by the average person. However, many people are exposed to low levels of chlorodibromomethane and bromoform in chlorinated water used for drinking, bathing, or swimming, and studies in animals indicate that chronic exposure to these chemicals may lead to increased risk of cancer. These effects and others of possible concern are discussed in greater detail below.

Death. Accidental overdoses of bromoform associated with the past use of bromoform as a sedative for whooping cough resulted in the death of a number of children in the early part of this century (Dwelle 1903; Kobert 1906; Roth 1904). The cause of death in these cases was usually marked depression of the central nervous system accompanied by respiratory or cardiovascular collapse. The amount of bromoform needed to cause death in humans is not known with certainty, but is probably about 300 mg/kg (Dwelle 1903; Roth 1904). No cases of human death from chlorodibromomethane are known, but studies in animals indicate that chlorodibromomethane and bromoform have roughly similar toxicity. On this basis, it seems likely that a similar acute dose of chlorodibromomethane (i.e. around 300 mg/kg) could also cause death in humans. Opportunities for exposure to lethal doses of either chemical are now remote.

Systemic Effects. The chief systemic effects recognized following exposure to chlorodibromomethane or bromoform are injury to the liver and the kidneys. These effects have been investigated mostly in animals exposed by the oral route, but there is limited data indicating that similar effects occur following inhalation exposure as well.

Typical effects in liver include increased liver weight, vacuolization, and fat accumulation. Effects in kidney are usually characterized by tubular degeneration and mineralization, leading to nephrosis and decreased renal function.

Oral dose levels leading to renal and hepatic effects in animals vary somewhat between chlorodibromomethane and bromoform, and also between species and sexes. In general, renal and hepatic effects are not apparent below doses of about 30 to 50 mg/kg/day, are rather mild at doses of 50 to 200 mg/kg/day, and are not marked until doses reach 250 mg/kg/day. Although data on hepatotoxic and nephrotoxic doses in humans are not available, it is reasonable to assume that the doseresponse relation in humans is roughly similar to that in animals.

Other systemic effects of chlorodibromomethane or bromoform appear to be minor or absent. No direct effects of oral exposure of animals to chlorodibromomethane or bromoform have been noted for the respiratory, cardiovascular, hematological or musculoskeletal systems, or on the skin or eyes. Some gastrointestinal effects (stomach nodules and ulcers) have been noted in rats, but these are probably due to a direct irritant action on the stomach, and are not likely to be of concern except at high doses that also produce hepatic and renal lesions.

Immunological Effects. Only one study (Munson et al. 1982) has investigated the effects of chlorodibromomethane and bromoform on the immune system, but the findings of this study indicate that short-term oral exposure of mice to doses of 125 mg/kg/day or higher can produce significant changes in both the humoral and cell-mediated immune systems. It is difficult to judge whether these changes are accompanied by a significant impairment in the overall functioning of the immune system, although data from one study (NTP 1988) indicated that chronic exposure to bromoform might decrease resistance to viral infection. The effect of chlorodibromomethane and bromoform on the immune system of humans has not been studied, but the data of Munson et al. (1982) indicate that this is an effect of potential concern.

Neurological Effects. Animal studies indicate that both chlorodibromomethane and bromoform possess anesthetic properties, and bromoform was previously used as a sedative in the treatment of whooping cough in children. In children, oral doses of around 15 mg/kg/day of bromoform typically produced only mild sleepiness, while doses of 150 mg/kg sometimes produced stupor or deep narcosis, usually accompanied by depressed respiration and erratic heartbeat. Airborne concentrations of bromoform leading to nervous system depression in humans are not known, but brief exposures of animals to high concentrations (≥29,OOO ppm) leads to deep sedation within minutes (Sax 1984). These depressant effects on the nervous system appear to be fully reversible both in animals and humans, but it is difficult to rule out the possibility of subtle but enduring neurological changes following narcotizing doses.

Developmental Effects. Studies in animals indicate that neither chlorodibromomethane nor bromoform have significant fetotoxicity or teratogenicity in animals exposed to oral doses up to 200 mg/kg/day, although some minor skeletal anomalies were noted at doses of 100 or 200 mg/kg/day (Ruddick et al. 1983). No studies of developmental effects in humans have been performed, but the animal data suggest that effects of this sort are not likely to be of concern at the levels typically encountered in the environment.

Reproductive Effects. Studies in animals indicate that oral exposure to either chlorodibromomethane or bromoform does not result in significant damage to reproductive organs in males or females (NTP 1985, 1988, 1989). Continuous exposure of mice to high doses of chlorodibromomethane in water caused a marked reduction in fertility (Borzelleca and Carchman 1982), but this was probably due to marked maternal toxicity. Lower doses (those that did not produce maternal toxicity) did not result in significant impairment of reproduction. While the effects of chlorodibromomethane or bromoform on reproduction have not been studied in humans, the data from animal studies suggest that this is not likely to be major concern at typical human exposure levels.

Genotoxic Effects. The genotoxicity of chlorodibromomethane and bromoform has been investigated in a number of studies, both in vitro (Tables 2-4 and 2-5) and in vivo (Table 2-6). The results of these studies are generally mixed and are occasionally inconsistent, perhaps because of variations in the efficiency of extrinsic or intrinsic metabolic activation of the parent compounds under test conditions. Still, a number of studies found evidence for both mutagenic and cytogenetic effects by both chlorodibromomethane and bromoform. The

TABLE 2-4. Genotoxicity of Bromoform *In Vitro*

End Point	Species (Test System)	Strain	Results: With Activation	Results: Without Activation	Reference
Prokaryotic organisms:					
Gene mutation	*Salmonella typhimurium* (desiccator system)	TA100	No data	+	Simmon et al. 1977
		TA1535	No data	+	
	S. typhimurium (plate incorporation assay)	TA98	-	-	Varma et al. 1988
		TA100	-	(+)	
		TA1535	-	-	
		TA1537	-	-	
		TA100	No data	-	Rapson et al. 1980
	S. typhimurium (preincubation procedure)	TA97	(+)	-	NTP 1988
		TA98	-, -, (+)	-, -, -	
		TA100	-, -, -	(+), -, -	
		TA1535	-, -, -	-, -, -	
		TA1537	-, -, -	-, -, -	
Eukaryotic organisms:					
Fish:					
Sister-chromatid exchange	Oyster toadfish leukocytes		No data	-	Maddock and Kelly 1980
Mammalian cells:					
Sister-chromatid exchange	Chinese hamster ovary cells	CHO-W-B1	-, -	-, (+)	Galloway et al. 1985
	Human lymphocytes		No data	+	Morimoto and Koizumi 1983
Chromosomal aberrations	Chinese hamster ovary cells	CHO-W-B1	-, -	-, (+)	Galloway et al. 1985
Trifluorothymidine resistance	Mouse lymphoma cells	L1578Y	+	+	NTP 1988

+ = positive result; - = negative result; (+) = marginally positive result. Results from two or more different contract laboratories are separated by commas.

TABLE 2-5. Genotoxicity of Chlorodibromomethane *In Vitro*

End Point	Species (Test System)	Strain	Results: With Activation	Results: Without Activation	Reference
Prokaryotic organisms:					
Gene mutation	*Salmonella typhimurium* (desiccator system)	TA100	No data	+	Simmon et al. 1977
	S. typhimurium (plate incorporation assay)	TA98	+	-	Varma et al. 1988
		TA100	+	-	
		TA1535	+	+	
		TA1537	+	+	
	S. typhimurium (preincubation assay)	TA98	-	-	Zeiger et al. 1987
		TA100	-	-	
		TA1535	-	-	
		TA1537	-	-	
Eukaryotic organisms:					
Fungi:					
Gene conversion	*Saccharomyces cerevisiae*	D7	-	+	Nestman and Lee 1985
Gene reversion	*S. cerevisiae*	XV185-14C	-	-	Nestman and Lee 1985
Mammalian cells:					
Sister-chromatid exchange	Human lymphocytes		No data	+	Morimoto and Koizumi 1983
	Human lymphocytes	CCRF-CEM	No data	+	Sobti 1984
	Rat liver cells	RL_4	No data	(+)	Sobti 1984
	Chinese hamster ovary cell		-	+	NTP 1988
Chromosomal aberrations	Chinese hamster ovary cell		-	-	NTP 1988

+ = positive result; - = negative result; (+) = marginally positive result.

TABLE 2-6. Genotoxicity of Chlorodibromomethane and Bromoform <u>In Vivo</u>

Chemical	End Point	Species (Test System)	Exposure Route	Results	Reference
Chlorodibromomethane	Mammalian systems:				
	Sister chromatid exchange	Mouse (bone marrow cell)	PO	+	Morimoto and Koizumi 1983
		Mouse (bone marrow cell)	IP	-	NTP 1988
	Chromosomal aberrations	Mouse (bone marrow cell)	IP	-	NTP 1988
Bromoform	Nonmammaliam systems:				
	Sex-linked recessive lethal	Drosophila melanogaster	Feeding	+	Woodruff et al. 1985
			Injection	-	Woodruff et al. 1985
	Reciprocal translocation	Drosophila melanogaster	Feeding	-	Woodruff et al. 1985
	Mammaliam systems:				
	Sister chromatid exchange	Mouse (bone marrow cell)	IP	+	NTP 1988
		Mouse (bone marrow cell)	PO	+	Morimoto and Koizumi 1983
	Chromosomal aberrations	Mouse (bone marrow cell)	IP	-	NTP 1988
	Micronucleus test	Mouse (bone marrow cell)	IP	+	NTP 1988

PO = oral; IP = intraperitoneal; + = positive result; - = negative result.

significance of these data are difficult to interpret with respect to human health risk, except that positive genotoxicity findings are consistent with a carcinogenic potential for these chemicals.

Cancer. Studies in animals indicate that both chlorodibromomethane and bromoform have carcinogenic potential. Chlorodibromomethane was found to increase the incidence of liver tumors (adenomas and/or carcinomas) in mice (NTP 1985), and bromoform was found to increase the frequency of intestinal tumors (adenomatous polyps and adenocarcinomas) in rats (NTP 1988). These findings are of special concern since many people are chronically exposed to low levels of these chemicals in chlorinated drinking water, and some epidemiological studies suggest that consumption of chlorinated drinking water may increase risk of cancer of the stomach, rectum, colon, and bladder (Cantor et al. 1987; Crump 1983; Kanarek and Young 1982; Marienfeld et al. 1986).

On the other hand, it should be noted that most of the carcinogenic responses in rats and mice exposed to chlorodibromomethane and bromoform were rather small, and that the weight of evidence for carcinogenicity was considered to be clear in only one case (intestinal tumors in female rats given bromoform). Also, the weight of epidemiological evidence for an association between ingestion of chlorinated water and increased cancer risk is not definitive (Cantor 1983; Crump 1983), and such an association (even if it were definitive) cannot provide direct evidence that either chlorodibromomethane or bromoform is carcinogenic in humans, since chlorinated water contains hundreds of other chemicals besides chlorodibromomethane and bromoform. Consequently, while exposure to low levels of chlorodibromomethane or bromoform in drinking water or from any other source is cause for concern, the relative contribution of these chemicals to human cancer risk remains to be resolved.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time biologic samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc and selenium). Biomarkers of exposure to chlorodibromomethane and bromoform are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are often not substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by chlorodibromomethane and bromoform are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE."

2.5.1 Biomarkers Used to Identify or Quantify Exposure to Chlorodibromomethane and Bromoform

The most straightforward means of identifying exposure to chlorodibromomethane or bromoform in a person is measurement of parent compound in blood or expired air. Sensitive and specific gas chromatographic-mass spectrophotometric methods available for this purpose are described in Section 6.1. Quantification of exposure is complicated by the relatively rapid clearance rate of these compounds from the body, both by exhalation and metabolic breakdown. Data are not available on clearance rates in humans, but in animals clearance of parent is nearly complete within 8 hours (see Section 2.3.4). Consequently, this approach is best suited for detecting recent exposures (within 1 to 2 days).

No data are available on blood or breath levels of chlorodibromomethane or bromoform in acutely exposed individuals. Background concentrations in people not exposed to chlorodibromomethane or bromoform except through chlorinated drinking water (see Section 5.4.2) are about 0.6 ppb (Antoine et al. 1986), while levels in expired breath are undetectable (Wallace et al. 1986a, 1986b). Although chlorodibromomethane and bromoform are lipophilic, they do not appear to accumulate in adipose tissue (Stanley 1986), so measurement of parent levels in this tissue is not likely to be valuable as a biomarker of exposure.

The principal metabolites of chlorodibromomethane and bromoform are CO_2, CO, Cl^- and Br^-. None of these metabolites are sufficiently specific to be useful as a biomarker of exposure. It is suspected that reactive intermediates formed during metabolism may produce covalent adducts with proteins or other cellular macromolecules (see Section 2.3.3), but these putative adducts have not been identified nor has any means for their quantification been developed.

2.5.2 Biomarkers Used to Characterize Effects Caused by Chlorodibromomethane and Bromoform

The most sensitive clinical sign of exposure to bromoform in humans appears to be sedation, and it is likely the same is true for chlorodibromomethane. However, generalized central nervous system depression is too nonspecific to be useful as a biomarker of effects from low-level exposure to chlorodibromomethane or bromoform. Studies in animals indicate the liver and the kidneys are also affected, resulting in fatty liver, increased serum enzyme levels, and nephrosis. Effects on liver and kidney can be evaluated using a variety of laboratory and clinical tests (CDC/ATSDR 1990), but these are also too nonspecific to be valuable in recognizing early effects caused by low level exposure to these two chemicals.

2.6 INTERACTIONS WITH OTHER CHEMICALS

It is well-known that exposure to alcohols, ketones, and a variety of other substances can dramatically increase the acute toxicity of halomethanes such as carbon tetrachloride (ATSDR 1989b) and chloroform (ATSDR 1989a). Several studies have been performed to determine if the toxic effects of chlorodibromomethane and bromoform are similarly affected by these agents.

Hewitt et al. (1983) found that pretreatment of rats with a single oral dose of acetone resulted in a l0- to 40-fold potentiation of the hepatotoxic effects of a single oral dose of chlorodibromomethane given 18 hours later. Similarly, pretreatment of rats for one to three days with chlordecone resulted in a 7- to 60-fold potentiation of the hepatotoxic effects of a single oral dose of chlorodibromomethane (Plaa and Hewitt 1982a, 1982b). In contrast, chlordecone pretreatment had relatively little potentiating effect on the hepatotoxicity of bromoform (Agarwal and Mehendale 1983; Plaa and Hewitt 1982a).

The mechanism by which chemicals such as acetone and chlordecone potentiate halomethane toxicity is not known, but it is generally considered that at least some of the effect is due to stimulation of metabolic pathways that yield toxic intermediates. If so, the findings above support the hypothesis that the toxicity of chlorodibromomethane is mediated at least in part by metabolic generation of reactive intermediates, but that metabolism is relatively less important in bromoform toxicity.

Harris et al. (1982) found that exposure of rats to a combination of bromoform and carbon tetrachloride resulted in more liver injury (judged by release of hepatic enzymes into serum) than would be predicted by the effects of each chemical acting alone. The mechanism of this interaction is not certain, but may be related to dihalocarbonyl formation and lipid peroxidation (Harris et al. 1982).

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

Studies of chlorodibromomethane and bromoform toxicity in animals reveal that there may be some quantitative and qualitative differences in susceptibility between sexes and between species (see Section 2.2). The basis for these differences is not known, but one likely factor is sex and species-dependent differences in metabolism (see Section 2.3.3). On this basis, it is reasonable to assume that there could be some differences in susceptibility between humans as a function of sex, race, or age. However, there are no studies that provide data on this point. Studies in animals (discussed in Section 2.6) also suggest that humans exposed to alcohols, ketones, or other drugs (e.g., barbiturates, anticoagulants) that influence halomethane metabolism might be more susceptible to the toxic effect of chlorodibromomethane and perhaps bromoform as well. Persons with existing renal or hepatic disease might also be more susceptible, since these organs are adversely affected by exposure to chlorodibromomethane and bromoform.

2.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (inconsultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of chlorodibromomethane and bromoform is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of chlorodibromomethane and bromoform.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.8.1 Existing Information on the Health Effects of Chlorodibromomethane and Bromoform

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to chlorodibromomethane and bromoform are summarized in Figures 2-5 and 2-6, respectively. The purpose of these figures is to illustrate the existing information concerning the health effects of chlorodibromomethane and bromoform. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs" information.

As shown in Figure 2-5, the toxicity of chlorodibromomethane has been reasonably well studied in animals exposed by the oral route, but there are no data in animals on inhalation or dermal toxicity, and there are no data in humans by any route. As shown in Figure 2-6, the oral toxicity of bromoform in animals has also been well studied. In addition, because of its use as an oral sedative in the early part of this century, there are some human data on the depressant effect of bromoform on the nervous system and on lethal doses, and there are also a few inhalation studies in animals. The dermal toxicity of bromoform has not been studied.

2.8.2 Identification of Data Needs

Acute-Duration Exposure. Limited data from humans indicate that one of the primary acute effects of ingestion of bromoform is sedation. This is supported by studies in animals, where both chlorodibromomethane and bromoform produced central nervous system depression following oral or inhalation exposure. Studies in animals indicate that hepatic and renal injury may also occur following acute oral or inhalation exposure, and these effects occur at lower doses than measurable central nervous system depression. Inhalation data are too sparse to define the threshold for these effects, but oral data are more extensive and do permit derivation of an acute MRL. Comparable data on hepatic or renal

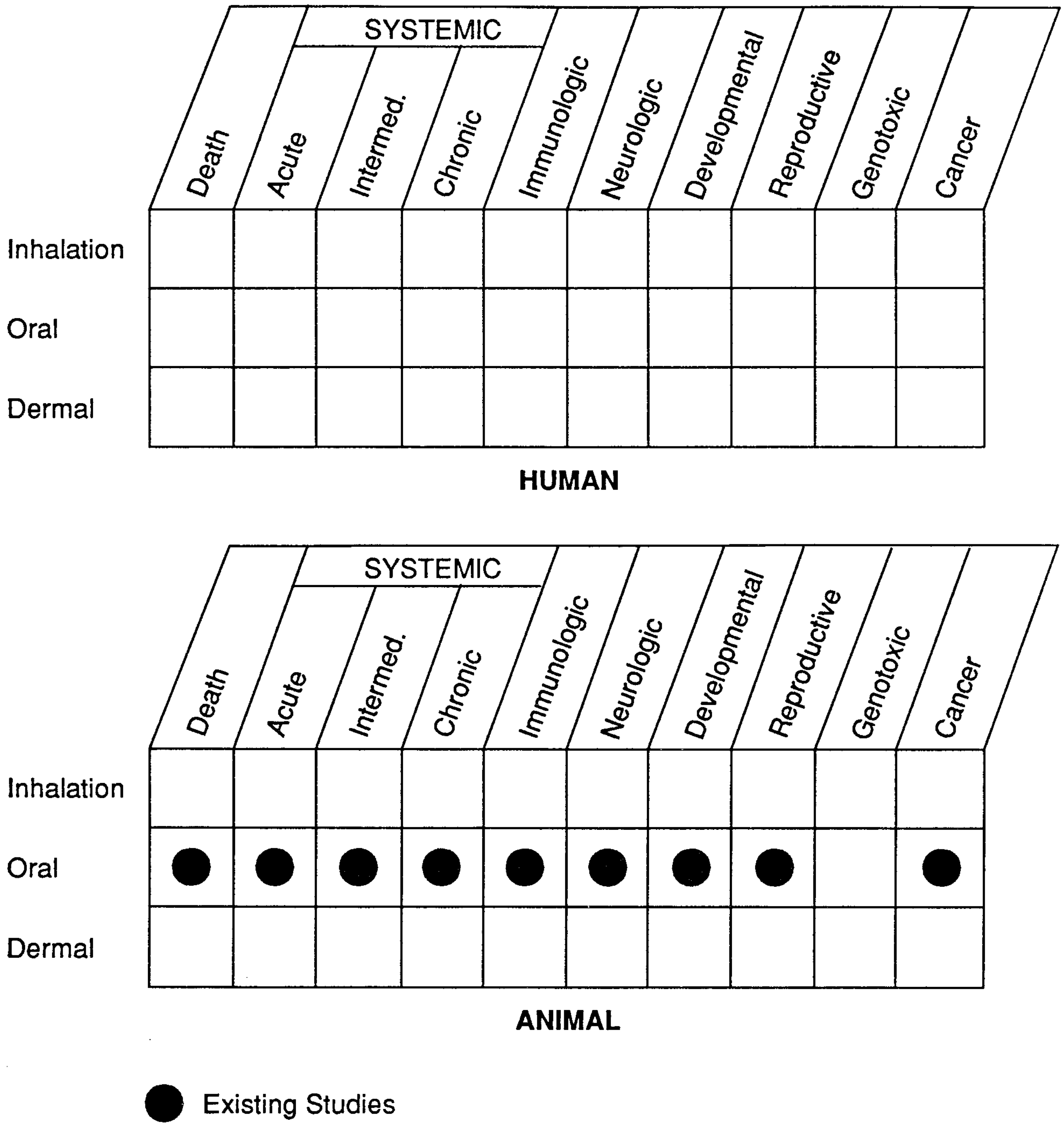

FIGURE 2-5. Existing Information on Health Effects of Chlorodibromomethane

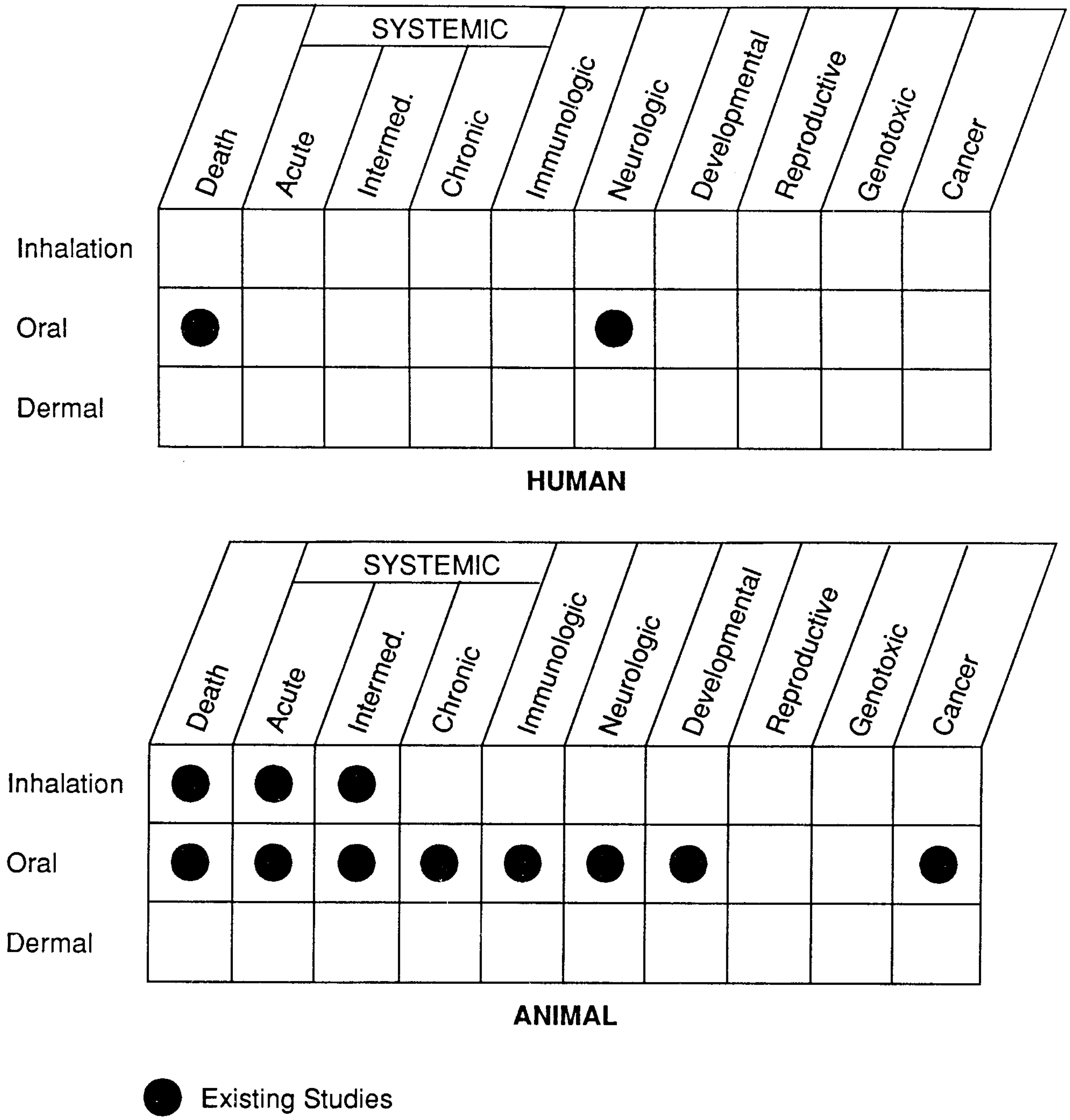

FIGURE 2-6. Existing Information on Health Effects of Bromoform

injury in humans are not available, but there is no evidence to suggest the same effects would not occur in humans. Additional studies on the short-term toxicity of chlorodibromomethane and bromoform would be valuable to further clarify the relative sensitivity of the nervous system, the liver, and the kidneys, and to define inhalation as well as oral dose-response curves more precisely. These data would be helpful since humans may be exposed to chlorodibromomethane and bromoform in air or water for brief periods following spills or releases at hazardous waste sites.

No data are available in humans or animals following dermal exposure to chlorodibromomethane or bromoform. Contact with concentrated solutions of these chemicals might be expected to produce effects similar to those following ingestion or inhalation, and might also result in skin or eye irritation. Studies on this would be useful, although contact with concentrated chlorodibromomethane or bromoform is considered extremely unlikely for members of the general population or residents near waste sites. Studies on the effects of dermal contact with lower levels of the compounds in water or soil would be valuable, since people might be exposed by these routes near waste sites.

Intermediate-Duration Exposure. The effects of intermediateduration oral exposure of animals to chlorodibromomethane and bromoform have been investigated in a number of studies, and the dose-response relation for the principal adverse effects (hepatic and renal toxicity) is fairly well defined. The data suggest the threshold for intermediate-duration renal and hepatic effects is similar to that for chronic exposure (see below), so an intermediate oral MRL has not been derived. Limited data indicate that intermediate-duration inhalation exposure to bromoform also leads to renal and hepatic injury in animals, but the data are too sparse to derive a reliable inhalation MRL. No intermediate-duration inhalation exposure data are available for chlorodibromomethane. Further studies on the intermediate-duration inhalation toxicity of these compounds would be valuable in assessing human health risks from airborne exposures near waste sites, although available data suggest exposures in air near such sites are likely to be low. As noted above, there are no data on dermal exposure, and studies on intermediate-duration dermal exposure to the compounds in water or soil would be useful in evaluating human health risk at waste sites.

Chronic-Duration Exposure and Cancer. The chronic oral toxicity of chlorodibromomethane and bromoform has been investigated in several studies, and the data are sufficient to identify hepatotoxicity as the most sensitive end point and to derive MRL values for both chemicals. However, in both cases, chronic oral MRLs are based on LOAELs for hepatotoxicity, so further studies to define the NOAELs would be helpful in reducing uncertainty in the MRL calculations. Chronic inhalation data are not available for either chemical, and would be useful, especially for chlorodibromomethane, since it is significantly more volatile (vapor pressure = 76 mmHg) than bromoform (vapor pressure = 5mmHg). In the absence of such data, extrapolation of observations from the oral route might be possible using appropriate toxicokinetic models. As noted above, no data exist for dermal exposure, and further studies (focusing on exposure in water or soil) would be valuable.

The carcinogenic effects of chronic oral exposure to chlorodibromomethane and bromoform have been investigated in well designed studies in both rats and mice, and the data suggest that both chemicals have carcinogenic potential. However, effects were limited or equivocal in some cases, so additional studies to strengthen the weight of evidence would be valuable. Of particular interest would be studies of the carcinogenic effects when exposure is via drinking water rather than by gavage, since drinking water is the most likely route of human exposure, and exposure by gavage (especially using oil as a medium) may not be a good model for this. Also of value would be studies on the mechanism of carcinogenicity, and the identity of carcinogenic metabolites. For example, studies on methylene chloride and other volatile halocarbons indicate that metabolism via a glutathione pathway may be important in carcinogenicity (e.g., Anderson et al. 1987; Reitz et al. 1989). Studies to determine if chlorodibromomethane or bromoform are metabolized by a similar pathway would be helpful in evaluating carcinogenic mechanism and risk.

Genotoxicity. There have been a number of studies that indicate chlorodibromomethane and bromoform are genotoxic, both in prokaryotic and eukaryotic organisms. However, a number of other studies have failed to detect significant genotoxic potential for these compounds. The basis for this inconsistency is not entirely obvious, but might be related to the efficacy of the test system to activate the parent compound to genotoxic metabolites. Further studies to define conditions under which these compounds are and are not genotoxic in vitro and in vivo may help clarify both the mechanism of genotoxicity and the relevance of this to human health risk. Studies on the genotoxic effects of chlorodibromomethane and bromoform on germ cells (sperm or ova) would also be valuable.

Reproductive Toxicity. No data are available on reproductive effects of chlorodibromomethane or bromoform in humans. However, chronic oral studies in rats and mice indicate that reproductive organs are not targets for either chlorodibromomethane (NTP 1985) or bromoform (NTP 1988). This is supported by direct studies showing no significant reproductive effects in mice following oral exposure to bromoform for 2 generations (NTP 1989). However, high doses that produce frank maternal toxicity may impair reproductive success (Borzelleca and Carchman 1982).

No data are available on reproductive effects following inhalation exposure. Based on the oral studies, it does not seem likely that effects would occur except at very high levels, but inhalation exposure studies to confirm this important point would be valuable.

Developmental Toxicity. Several studies in animals exposed by the oral route indicate that neither chlorodibromomethane nor bromoform have marked teratogenic potential (Borzelleca and Carchman 1982; Ruddick et al. 1983). However, ingestion of bromoform did appear to increase the frequency of several skeletal abnormalities in fetuses. Additional oral studies on the developmental effects of both bromoform and chlorodibromomethane in animals would be valuable to determine whether these skeletal abnormalities are produced consistently, and whether they lead to significant adverse effects in the neonate. If so, then similar studies by the inhalation route would also be valuable to define safe inhalation levels for developmental effects.

Immunotoxicity. The immunotoxic effects of chlorodibromomethane and bromoform have been investigated in one 14-day oral study (Munson et al. 1982). That study indicated both chemicals can lead to changes in several immune cell-types in mice. Similar studies in other species would be valuable in determining if this is a common response. In addition, longer duration studies and tests of the functional consequence of these changes (e.g., resistance to infectious disease) would be especially valuable in assessing the biological significance of these effects. If these studies indicate the immune system is a target, then similar studies by inhalation exposure would also be valuable.

Neurotoxicity. Numerous studies, both in humans and animals, reveal that central nervous system depression is a rapid effect following either oral or inhalation exposure to bromoform, and more limited data indicate that chlorodibromomethane also causes this effect. While central nervous system depression appears to be reversible within a short time after exposure ceases, the possibility of permanent neurological damage from high doses has not been thoroughly studied. Histological studies by NTP (1985, 1988) indicate that sub-depressant doses of chlorodibromomethane and bromoform do not lead to detectable histological changes in the brain, but similar data are not available following narcotizing doses. In addition to histological studies, functional studies capable of detecting lasting neurological changes would be valuable. One study of this sort (Balster and Borzelleca 1982) indicates that both chlorodibromomethane and bromoform can cause some behavioral changes at high doses. Further studies along these lines, perhaps employing more sensitive tests of electrophysiological or neurobehavioral changes, would be helpful in determining if this is an effect of concern to exposed humans.

Epidemiological and Human Dosimetry Studies. No epidemiological or human dosimetry studies are currently available for chlorodibromomethane or bromoform. Since only very small quantities of these chemicals are produced or used in this country (see Chapter 4), it does not seem likely that a sufficiently large subpopulation of exposed workers exists to serve as the basis for a meaningful epidemiological study. Epidemiological studies of populations exposed to low levels of chlorodibromomethane and bromoform in chlorinated drinking water cannot provide specific data on the human health risks of chlorodibromomethane or bromoform, since chlorinated drinking water contains hundreds of different contaminants.

Biomarkers of Exposure and Effect. The only known biomarker of exposure to chlorodibromomethane or bromoform is the level of parent compound in blood or in expired air. However, data on blood or breath levels in humans following acute exposure are lacking, due to the rarity of such events. Since both chlorodibromomethane and bromoform are rapidly cleared from the body by exhalation or metabolism, measurements of parent compounds in blood or breath are likely to be useful only for a short-time (1-2 days) after an exposure. Monitoring of humans continuously exposed to the trace levels normally present in chlorinated water reveal very low to nondetectable levels in blood or expired air. The main metabolites of these compounds (CO_2 CO, Cl^-, Br^-) are not sufficiently specific to be useful for biomonitoring of exposure. Identification of stable and specific biomarkers of exposure (e.g., halomethyl adducts) would be valuable in evaluating the exposure history of people around waste sites and other sources where above-average levels might be encountered.

No specific biomarkers of chlorodibromomethane or bromoform-induced effect are known. Neurological, hepatic and renal effects caused by these chemicals can be detected by standard clinical or biochemical tests, but abnormal function in these tissues can be produced by a number of common diseases in humans, so detection of abnormal function is not proof that the effect was caused by chlorodibromomethane or bromoform. Efforts to identify more specific and sensitive biomarkers of chlorodibromomethane and bromoform-induced effects would be useful, especially biomarkers (e.g., specific DNA adducts) that might be predictive of carcinogenic risk.

Absorption, Distribution, Metabolism, Excretion. Limited data indicate that chlorodibromomethane and bromoform are rapidly and efficiently taken up from the gastrointestinal tract, but further studies to confirm and refine available estimates would be valuable. Toxicokinetic studies to date have generally employed exposure by gavage in corn oil, so studies involving exposure via an aqueous vehicle would be especially valuable. No toxicokinetic data exist for inhalation exposure, so quantitative estimates of the inhalation absorption fraction, tissue distribution, and excretion rate would be beneficial. Also, data on dermal absorption would be helpful, especially from soil or from dilute aqueous solutions, since this is how humans are most likely to experience dermal contact near waste sites.

The pathways of chlorodibromomethane and bromoform metabolism have been investigated in several laboratories, but quantitative data on the amount of chemical passing through each pathway are limited, and the chemical identity of products appearing in urine has not been studied. Of particular interest would be studies which seek to clarify the role of metabolism in toxicity, the mechanism by which metabolites and adducts lead to toxic effects, and the importance of protective mechanisms such as cellular antioxidants. This would include careful dose-response studies to determine if either activating or protective pathways are saturable.

Comparative Toxicokinetics. Available toxicity data indicate that target tissues of chlorodibromomethane and bromoform are similar in humans, rats and mice. Limited data suggest that effect levels are generally similar across species, but some distinctions are apparent. Toxicokinetic studies have revealed differences between rats and mice regarding metabolic patterns and clearance rates and these might underlie the differences in toxicity between tissues, sexes, and species. Additional comparative studies in animals, with special emphasis on differences in metabolism, would be useful in understanding these differences, and in improving inter-species extrapolation. In addition, in vitro studies of metabolism by human liver cells would be valuable in determining which animal species has the most similar pattern of metabolism and is the most appropriate model for human toxicity. Data from studies of this sort could then be used to support physiologically-based toxicokinetic models.

2.8.3 On-going Studies

Table 2-7 summarizes two on-going research projects on the health effects of chlorodibromomethane or bromoform. When completed, these studies may be expected to provide valuable new data on several topics, including reproductive, developmental, and carcinogenic effects of chlorodibromomethane and bromoform. In addition, the U.S. Department of Human Health Services is sponsoring an on-going study (the National Health and Nutrition Examination Survey) which will provide data on levels of bromoform and chlorodibromomethane in blood of humans at numerous locations across the United States.

TABLE 2-7. Summary of On-going Research on the Health Effects of Chlorodibromomethane or Bromoform

Principal Investigator	Affiliation	Research Description	Spons. Agency
H. Lilja	Mason Research Inst., Worcester, Massachusetts	Subchronic and chronic toxicity and carcinogenicity of chlorodibromomethane in mice and rats, using various routes of exposure	NIEHS
	Division of Toxicology National Inst. of Hygienic Sciences Tokyo, Japan	Carcinogenicity study on chlorodibromomethane and bromoform	Government of Japan

NIEHS = National Institute of Environmental Health Sciences.